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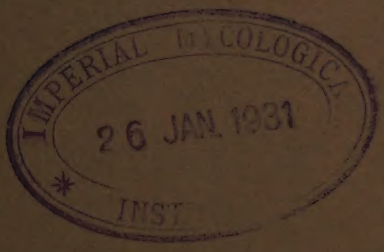

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STUDIES ON THE PROPERTIES OF THE BEAN-MOSAIC VIRUS

T. G. FAJARDO[1]

The determination of the properties of different plant viruses now occupies an increasingly important position with respect to their classification. The properties of the ordinary tobacco-mosaic virus and that of cucumber mosaic, for example, are now well known (7, 4), but, up to the present, nothing has been reported concerning certain properties of the bean-mosaic virus. This may be because bean mosaic is difficult to transmit artificially, a difficulty frequently encountered by the earlier workers. A method of artificial inoculation has been developed by the writer (5), however, that has given a fairly high percentage of infection and has made possible a preliminary study of the properties of this virus. The investigation has been conducted in relation to the following phases: (1) Effect of aging on the virus *in vitro,* (2) effect of dilution, (3) resistance to alcohol treatments, (4) resistance to drying in plant tissues, (5) thermal death point, (6) effect of dry heat on the virus in infected seeds, (7) effect of freezing, and (8) filterability through bacterium-proof filters.

METHODS

The methods followed in these studies were uniform unless otherwise stated. The bean-mosaic virus was obtained in plant extract either by grinding up mosaic bean plants in a sterilized mortar with sterile sand or by passing diseased material through a fine meat chopper. The juice was then separated from the macerated tissues by squeezing it through cheesecloth. After the various treatments, the juice was artificially inoculated to young healthy seedlings of the variety Rogers Stringless Green Pod or Refugee 1000–1, both of which are late susceptible varieties. The inoculation was performed by rubbing or mutilating the two simple leaves of the plants with a small piece of cheesecloth dipped into the juice, to which a small quantity of sterilized sand had been added. The control plants were inoculated by the same method with a portion of the same but nontreated plant extract. The plants were kept in a greenhouse at a temperature of 24–28° C. for 25 days. Under ordinary greenhouse conditions, infected plants develop typical mosaic symptoms 15 to 20 days after inoculation.

RESULTS

(1) *Effect of aging on the virus in vitro.* The results of a series of experiments have shown that the virus of bean mosaic loses its power of

[1] The writer is indebeted to Professor L. R. Jones, under whose encouragement the work was pursued, and to Drs. J. Johnson and I. A. Hoggan, who kindly read and criticized the manuscript during its preparation.

infection rapidly in plant extract. Undiluted juice, whether allowed to stand in bulk or in 5 c.c. portions at room temperatures, failed to cause infection after 24 hours. Juice diluted with sterile water under the same greenhouse conditions loses its infectiousness more rapidly. Dilutions of 1 part of plant extract to 10 parts of water remained infectious after 14 hours, while dilutions of 1 to 50 and 1 to 100 failed to produce infection after this time. The percentage of infection decreased markedly with increase in dilution and duration of aging.

(2) *Effect of dilution.* The results of four experiments indicate that the bean-mosaic virus loses its infectious properties readily on dilution with distilled water, without aging. A low percentage of infection was obtained when the virus was diluted in plant extract to 1 to 1000. No infection was secured at dilutions higher than 1 to 1000.

(3) *Resistance to alcohol treatments.* The virus of bean mosaic is very sensitive to alcohol. A low percentage of infection was obtained after treatment of the virus with 50 per cent alcohol at room temperatures for less than five minutes, while no infection resulted after treatment with 75 per cent alcohol or higher strengths for the same length of time. No infection was obtained after treatment with 25 per cent alcohol for 30 minutes at room temperature.

(4) *Resistance to drying in plant tissues.* The bean-mosaic virus is also very sensitive to drying and loses its infectious properties rapidly on drying. Cut young mosaic plants were allowed to wilt or dry at room temperature for various lengths of time, each sample being weighed at the beginning and end of the period. The loss in weight was then replaced by addition of distilled water, the plants macerated, and the extract obtained inoculated immediately to young plants. Table 1 shows the results of two such experiments and indicates that the virus was still infectious in plants that had been allowed to dry for 48 hours. No infection was secured from plants after drying 72 hours. Young mosaic plants kept turgid in an ice box, however, were still infectious after 72 hours. The virus, therefore, lives longer in plant tissues than in plant extract but becomes inactivated when the tissues dry out.

(5) *Thermal death point.* The bean-mosaic virus has a relatively low thermal death point. Five and 10 c.c. portions of mosaic plant extract were heated for 10 minutes in thin-wall test tubes in a water bath. After heating at 44–46° C., a single infection resulted in 10 plants inoculated, while no infection obtained after heating at 55–56° C. The thermal death point for the virus of bean mosaic therefore appears to lie between 44° C. and 56° C., probably close to 46° C.

(6) *Effect of dry heat on the virus in infected seeds.* Several tests with mosaic seeds of the varieties Rogers Stringless Green Pod and Refugee

TABLE 1.—*Resistance of the bean-mosaic virus to drying at room temperature*

Inoculum	Duration of aging	Loss of weight	Plants infected of 5 inoculated
	Hours	*Per cent*	*No.*
Series I			
1 (Control)	0	0.0	5
2	4	35.7	5
3	12	53.6	4
4	24	66.7	1
5	48	80.0	1
6	72	82.6	0
7	96	86.4	0
Series II			
1 (Control)	0	0.0	4
2	16	50.0	4
3	24	65.0	3
4	48	84.0	1
5	72	87.5	0
6	96	90.0	0

1000–1, heated 12 and 24 hours, respectively, at various temperatures in thermo-regulated ovens, showed that the virus could withstand higher temperatures in dry seeds than in plant extract. A certain number of mosaic seedlings were obtained from seeds heated at 65–66° C. for 24 hours, while seeds heated at 80° to 85° C. failed to germinate. Water extracts of the seeds heated at 80°–85° C. gave no infection when inoculated to young bean plants. These results, summarized in table 2, show that the highest temperatures which allow of subsequent germination do not destroy the virus in the seed. This is in agreement with the conclusions reached by Reddick and Stewart (10).

(7) *Effect of freezing.* Freezing undiluted, freshly expressed juice of mosaic plants at temperatures of –7° to –10° C. did not appear to affect the viability of the virus. The percentage of infection was found to decrease with the duration of freezing, until after 24 hours no infection resulted. The results, however, were similar to those obtained when testing at room temperature the effect of aging on the virus. It is, therefore, believed that the freezing, itself, did not cause inactivation of the virus.

(8) *Filterability through bacterium-proof filters.* The bean-mosaic virus, as far as could be determined, failed to pass through any grade of Berkefeld filter at a reduced pressure. In these experiments, inoculation of the first 5 to 10 c.c. of the filtrate passing through a grade V, coarse Berkefeld filter gave no infection. The nonfiltered juice was found to be still highly infectious and gave 100 per cent infection.

TABLE 2.—*The effect of dry heat on the bean-mosaic virus in infected seeds*

Variety	Time and temperature of heating	Seeds planted	Germination	Mosaic infection
		No.	*Per cent*	*Per cent*
	12 Hours			
	24° C.	20	95	58
Rogers	44–46° C.	20	60	67
Stringless	65–68° C.	40	80	59
Green Pod	80–85° C.	20	0	0
	85–90° C.	20	0	0
	24 Hours			
	24° C.	20	75	73
	44–46° C.	20	85	71
	65–66° C.	40	90	72
	80–85° C.	20	0	0
	85–90° C.	20	0	0
	12 Hours			
	24° C.	20	90	94
Refugee	44–46° C.	20	85	88
1000–1	65–66° C.	40	58	82
	80–85° C.	20	0	0
	85–90° C.	20	0	0
	24 Hours			
	24° C.	20	95	84
	44–46° C.	20	90	83
	65–66° C.	40	25	60
	80–85° C.	20	0	0
	85–90° C.	20	0	0

A COMPARISON OF THE PROPERTIES OF THE BEAN-MOSAIC VIRUS WITH THOSE OF CERTAIN OTHER VIRUSES

These results indicate that bean mosaic is caused by a specific virus, distinct from other viruses that have been similarly studied, such as the virus of tobacco mosaic, of cucumber mosaic, and of potato-crinkle mosaic. This is readily seen from table 3. While the tobacco-mosaic virus may remain infectious in plant extract for 1 or more years (3), the bean-mosaic virus apparently retains its infectiousness only about 20–24 hours, in this respect resembling more closely the viruses of cucumber mosaic and potato-crinkle mosaic, which lose their power of infection in 24–48 hours and in 24 hours, respectively (4, 8). The virus of bean mosaic retains its infectious nature in dried mosaic tissues between 2 and 3 days; that of tobacco mosaic for more than 1½ years (1); and that of cucumber mosaic (4), less than 10 days.

Again, while the bean-mosaic virus gives no infection at dilutions higher than 1–1000, the tobacco- and cucumber-mosaic viruses are still infectious at dilutions of 1 to 10,000 (2, 4). On the other hand, the crinkle-mosaic virus produces little infection at dilutions above 1 to 10 (8) and is, in this respect, more sensitive than the bean-mosaic virus.

The bean-mosaic virus is relatively sensitive also to alcohol. No infection was obtained after treatment with 25 per cent alcohol for 30 minutes, while the tobacco-mosaic virus resists 50 per cent alcohol for several days and 80 per cent alcohol for half an hour or less (3). Johnson (7) obtained no infection with the cucumber-mosaic virus after treatment with 50 per cent alcohol for 1 hour.

The viruses also differ in their thermal death points, that of bean mosaic lying between 44° and 56° C., probably close to 46° C., that of tobacco mosaic being about 90° C. (9), that of cucumber mosaic 75° C. (4), and that of potato-crinkle mosaic 43° C. (8).

No success was obtained in attempts to filter the bean-mosaic virus through coarse Berkefeld filters, the virus being in this respect comparable to that of crinkle mosaic. The tobacco-mosaic virus has been shown by Iwanowski (6) and others to pass readily through bacterium-proof filters, and the cucumber-mosaic virus has been shown by Doolittle (4) to pass through a medium Berkefeld filter.

TABLE 3.—*Comparison of the properties of the bean-mosaic virus with those of the viruses of potato, tobacco, and cucumber mosaics*

Property of virus	Source of virus			
	Bean mosaic	Potato "crinkle mosaic"[a]	Tobacco mosaic[b]	Cucumber mosaic[c]
Resistance to aging *in vitro*	20–24 hrs.	24 hrs.	1 or more yrs.	24–48 hrs.
Tolerance to dilution	1–1000	1–10	1–10,000	1–10,000
Resistance to alcohol	25% for 30 min.	——	80% for 30 min.	50% for 1 hr.[a]
Resistance to aging in dried plant tissues	48–72 hrs.	——	1 or more yrs.	Less than 10 days
Thermal death point	Probably close to 46° C.	43° C.	90° C.[d]	75° C.
Filterability through coarse Berkefeld filter	Not filterable	Not filterable	Filterable	Filterable

[a] Determination by Johnson.
[b] Determination by Allard except as noted.
[c] Determination by Doolittle except as noted.
[d] Determination by Mulvania.

SUMMARY

(1) The virus of bean mosaic differs in its properties from the viruses of tobacco mosaic, cucumber mosaic, and potato-crinkle mosaic.

(2) The bean-mosaic virus retains its infectiousness for 20–24 hours in plant extract, when aged at room temperatures. In diluted juice, the virus loses its infectiousness sooner than in nondiluted juice.

(3) At a dilution of 1–1000 with distilled water, mosaic-plant extract gives a low percentage of infection, but at higher dilutions no infection has been obtained.

(4) The virus is inactivated by treatment with 25 per cent alcohol for 30 minutes and with 75 per cent alcohol for less than 5 minutes.

(5) The virus is very sensitive to drying in plant tissues, since infection was obtained from mosaic seedlings that had been allowed to dry at room temperatures for 72 hours.

(6) The thermal death point of the virus appears to lie between 44° and 56° C., probably close to 46° C.

(7) The virus is able to resist higher temperatures when in the seed than in expressed juice. It was not destroyed at temperatures that did not destroy the germinating capacity of the seed.

(8) Freezing apparently does not affect the viability of the virus.

(9) The virus was not found to pass through any grade of Berkefeld filter.

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